

ANDREW GORBA:



EXHIBIT
1

Received 60cc cord blood (w/ CPD)
PE mono 30%
T cells 70%

added 15ml 1xPBS to bring to incubation
volume of 75ml.

Added 1.5ml antibody (12.8)
incubated 25min.

Primed CellPro "separate".

spin cells. Resuspended in 1x PBS to
a final volume of 300cc in bag.

Ran through column.

Unadsorbed portion → spun down.
and consolidated in 1x PBS
for incubation.

75ml for incubation (added HSA)
1.5ml antibody (12.8) 25min. Spun down
following incubation. Rpd to vol. of 300cc in bag.
Ran through 2nd column.

stem cell portions from Runs 1 & 2
were combined (after counts, and
samples removed for staining)

total cells 2.8×10^6 for transduction

BBMM +

1L3 SANDO

WEN

1L6 SANDO

WEN

SLF AUBRE

WEN

final concn
will be dil
cells are
concentrated

BBMM: FB
BS

2.8×10^6 cells
Put in

BBMM + 31615CF (for 500ml of media)

123 SANDOZ * Y0230892 stock at 150ug/ml
want final: 20ug/ml x 2 ... 20ug

133ul add

126 SANDOZ * Y0450392 stock at 150ug/ml
want final: 50ug/ml x 2 ... 50ug

333ul add

SCF ANTIBEN * 1509F2 stock at 1.5mg/ml = 1500ug/ml
want final: 100ug/ml x 2 ... 100ug

167ul add

final concentrations are doubled since the media
will be diluted 1:2 w/ viral supernatant.
Cells are therefore incubated with the correct
concentrations.

BBMM: FBS Gemini Lot# A26003H
BSA #115

2.8×10^6 cells want final: 5×10^4 cells.

Put in 2 T75 30ml each: 15ml B365 051193
15ml LPSU^{G7} Lot# 53

+ protamine sulfate 240ul
of 1:10 diluted 50

in down
P 3000 in bag.

42
and

Cord Blood cells pre processing:

CFUs

SET 143

Start:

Plate #	Sample	# Cells	# ul/ml media
- G418	1ab	5×10^4	50
+ G418	2ab		50
- G418	3ab	1×10^5	100
+ G418	4ab		100

adsorb
fractionadsorb
fraction

CFUs Post transduction: SET 144

plate #	# Cells	# ul
- G418	500	7
	1000	14
	2000	28
+ G418	500	7
	1000	14
	2000	28

(yields)
adsorb

COUNT:

$$\bar{x} = 34$$

$$\times 2 \times 10^4 = 6.8 \times 10^5 \text{ Cfu/ml}$$

$$\times 5.5 \text{ ml} = 3.7 \times 10^6 \text{ C}$$

Reinforced on 5/15/93
No transduced stem cells

G418

-

+

7ab
8ab1000
200020
40adsorb
fraction from
medium

Start:

 $5 \times 10^8 \text{ c}$ PRE
 0.71% Post ab
 0.22% $*34+ = 3.6 \times 10^6 \text{ c}$ $= 1.1 \times 10^6 \text{ c}$ cell/ml media30
30
20
100adsorbed
fraction #1: $2 \times 10^6 \text{ c}$ FL1 FL2 gate
 31.94% FL1 FL2 gate
 20.81% $*34+ = 0.64 \times 10^6 \text{ c}$ $= 0.42 \times 10^6 \text{ c}$ adsorbed
fraction #2: $0.8 \times 10^6 \text{ c}$ 2.46% 5.80% $\pm 34+c = 0.02 \times 10^6 \text{ c}$ $0.05 \times 10^6 \text{ c}$

(yields)

adsorbed #1:

PRE & FL1/FL2 gate
 $\frac{0.64 \times 10^6 \text{ c}}{3.6 \times 10^6 \text{ c}} = \boxed{17.8\%}$ PRE & FL1/FSC gate
 $\frac{0.42 \times 10^6 \text{ c}}{3.6 \times 10^6 \text{ c}} = \boxed{11.7\%}$ post ab & FL1/FL2 gate
 $\frac{0.64 \times 10^6 \text{ c}}{1.1 \times 10^6 \text{ c}} = \boxed{58.2\%}$ Post ab & FL1/FSC gate
 $\frac{0.42 \times 10^6 \text{ c}}{1.1 \times 10^6 \text{ c}} = \boxed{38.2\%}$

adsorbed #2:

PRE & FL1/FL2

PRE & FL1/FSC

removed from
medium

medium

ZACHARY RIGGINS:

5/14/93

REC'D 200 cc COLD BLOOD

PRE: $\frac{morb}{109}$ \checkmark $\frac{poly}{109}$

$$218 \times 50 = 10.9 \times 10^6 \text{ clml} \\ \times 200 \text{ ml} = 2.2 \times 10^9 \text{ C} \quad \text{start}$$

Added 3 vials (4.5 ml) 12.8 ab.
inc. 25 min.

Spindown. Rsp'd. in 1x PBS to 300 ml
in bag.

Ran through column:

spin down unadsorbed fraction for 2nd ab
incubation.

Spin stem cell fraction to Rsp'd. in
smaller volume for count.

COUNTS:

unadsorbed

$\frac{morb}{67}$ \checkmark $\frac{poly}{102}$

$$1109 \times 50 \times 10^3 \\ = 8.5 \times 10^6 \text{ clml} \times 225 \text{ ml} \\ = 1.9 \times 10^9 \text{ C}$$

stem

$\frac{morb}{172}$ \checkmark $\frac{poly}{16}$

$$188 \times 2 \times 10^4 \\ = 3.8 \times 10^6 \text{ clml} \times 5.5 \text{ ml} \\ = 20.7 \times 10^6 \text{ C}$$

incl
12.8
3pc
RPT
Ran

con

1
0
3

3.
=

per
free

con

26x1
was
= 2 =

13 fl.

LA 50

incubated unadsorbed fraction w/ 4.5 ml
 b.8 ab. for 25 min.
 spun down.

Put in 300ml in bag (w/ 1x PBS)
 Ran through 2nd column.

counts:

unadsorbed
monos polys

30 33
 $63 \times 52 \times 10^3$

$3.15 \times 10^4 \text{ cpm} \times 600 \text{ ml}$
 $= 1.9 \times 10^9 \text{ c}$

↓

stem

monos polys

58 41
 $62 \times 2 \times 10^4$

$= 1.2 \times 10^6 \text{ cpm}$
 $\times 5 \text{ ml} = 6 \times 10^6 \text{ c}$

percolled/ficoll
 free \Rightarrow LWT(2)

combined stem cell fractions

$26 \times 10^6 \text{ c}$ for transduction
 want final $[T] = 5 \times 10^4 \text{ c/ml}$
 $= 2 = 200 \text{ ml sup}$
 260 ml media

520 ml total

13 flasks 40 ml/flask

20 ml sup

20 ml media (B365)

+ 300 ml protamine sulfate

LASN sup 539 (bottles 18/19)

$\times 10^4$
 ml $\times 5.5 \text{ ml}$

CFUs:

5/4/45

PRE

Plate #	Sample	# Cells	# ul
lab	(-G418)		
2ab	PRE + G418	5×10^4	5
	↓ (+G418)	↓	5

BBMM + 31615CF:

1L3 Sander #y0230292

1L6 Sander #y0450392

SCF AMGW #150952

Took sample to micro for sterility ✓
 each day of transduction pd.
 Stat Gram stain done (negative)
 before cells were given to baby.

5/15/93 4pm 2nd transduction:

Spun cells down from each flask
 Respd in fresh media & LASN sup
 added Protamine sulfate

5/16/93 3rd transduction 330pm.
 Repeated above.

5/15

COW

Dx

Rex

Fos

OG

+G4

OG418

+G4

5/17/93 cells washed 4x
 3x in 1x PBS + Pls
 last wash in RPMI (no pls)

COUNT: 60×10^6 c

$$\bar{x} = 15 \times 10 \times 10^4 = 15 \times 10^9$$

$$\times 40 \text{ ml} = 60 \times 10^6 \text{ c}$$

Put in 5cc into 10cc syringe

Reinfused on 5/17/93 (UCSF)

Post-trans. CPUs: 59746

sample plate #		# cells	# ul
OG418	1ab	500	4
↓	2ab	1000	8
↓	3ab	2000	16
+G418	4ab	500	4
↓	5ab	1000	8
↓	6ab	2000	16
OG418	7ab	1000	24
+G418	8ab	1000	24

ry ✓
 wire)
 > baby
 lion
 in flask
 SN supe

30pm.